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A pictorial key to differentiate the recently detected exotic *Haemaphysalis longicornis* Neumann, 1901 (Acari, Ixodidae) from native congeners in North America

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Abstract

Until recently, only two haemaphysaline species, *Haemaphysalis chordeilis* (Packard, 1869) and *Haemaphysalis leporispalustris* (Packard, 1869), were known to occur in the United States, and neither was considered to be of significant medical or veterinary importance. In 2017–2018 established populations of the Asian longhorned tick, *Haemaphysalis longicornis* Neumann, 1901, were detected in the eastern US for the first time. *Haemaphysalis longicornis* has the potential to be a significant threat to human and animal health, and the urgent need to determine the full extent of its distribution and host range requires availability of a straightforward and practical guide to differentiate it from native species. We created a pictorial dichotomous key to all stages of *Haemaphysalis* spp. known to occur in North America with scanning electron photomicrographs of all *H. longicornis* life stages, including rarely seen males, to aid researchers in differentiating these species. The largely Neotropical species *Haemaphysalis juxtakochi* Cooley, 1946, with established populations in Mexico and sporadic detections in the US on migrating birds is also included.

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Keywords
Asian longhorned tick, haemaphysaline fauna, dichotomous key, scanning electron microscopy, invasive species

Introduction

Only two native species of *Haemaphysalis* ticks are known to occur in the United States: the rabbit tick, *Haemaphysalis leporispalustris* (Packard, 1869) and the bird tick, *Haemaphysalis chordeilis* (Packard, 1869) (Keirans and Litwak 1989). *Haemaphysalis leporispalustris* is common and widespread in North America, and is frequently collected from lagomorphs (rabbits and hares) (Bishopp and Trembley 1945). Its full distribution extends from Alaska and Canada southward to Argentina (Kohls 1960, Guglielmone et al. 2003). The agents of tularemia (*Francisella tularensis*) and of Rocky Mountain spotted fever (*Rickettsia rickettsii*) have been isolated from this tick (Eremeeva et al. 2018) although its role, if any, as a vector of human disease appears to be minor. *Haemaphysalis chordeilis* is far less often collected but nonetheless assumed to have a broad distribution in North America, based on sporadic avian records spanning the US and southern Canada (Bishopp and Trembley 1945, Kohls 1960, Lindquist et al. 2016). Because these two species have historically been considered specialists on rabbits and birds, respectively, and are not significant pests of humans or domestic animals, relatively little attention has been paid to their ecology and geographical distribution.

In 1993, a single specimen of the Central/South American species *Haemaphysalis juxtakochi* Cooley, 1946 (*Haemaphysalis kochi* Aragão, 1908 is a junior synonym) was detected in Ohio, USA, on a white-tailed deer at a deer-checking station (Keirans and Restifo 1993). While the current northern limit of this species' distribution appears to be Mexico, immatures may occasionally be brought into the US by northward migrating birds (Mukherjee et al. 2014). At present there is no indication that such encounters are common or that the species has become established in the US. Adult *H. juxtakochi* are chiefly parasites of deer (Kohls 1960, Guglielmone et al. 2005) and may be able to transmit some species of *Rickettsia* (Souza et al. 2018).

In 2017, established populations of the East Asian/Australasian species *Haemaphysalis longicornis* Neumann, 1901, were discovered in New Jersey (Rainey et al. 2018) and subsequently throughout a large part of the eastern US, including Arkansas, Connecticut, Maryland, New York, North Carolina, Pennsylvania, Virginia, and West Virginia (Beard et al. 2018). This species, native to East Asia and invasive in Australia/New Zealand, is associated with disease transmission to humans in the former region (e.g., Zhuang et al. 2018) and is a serious pest of livestock in the latter (Heath 2016). Invasive populations of this species appear to be parthenogenetic, which may facilitate their establishment and spread (Heath 2013). As a result, there is now much concern over this species' potential effect on human and animal health in the United States, and studies are under way to clarify its current geographic range and preferred host species.

In order to study potential impacts of *H. longicornis* on North America, a critical first step is being able to differentiate this species from co-occurring *Haemaphysalis* spp. Here we present scanning electron photomicrographs of all stages of *H. longicornis*, as well as a simple, usable dichotomous key to differentiate the four *Haemaphysalis* species that may be encountered in North America: *H. leporispalustris*, *H. chordeilis*, *H. longicornis*, and *H. juxtakochi*. While the rarity of *H. juxtakochi* detections in the US does not suggest that this species will often be sympatric with *H. longicornis*, we feel it is important to include it in our key for three reasons: (1) climate change is predicted to alter the distribution of many tick species (Ostfeld and Brunner 2015), therefore the distribution of *H. juxtakochi* may one day shift north of Mexico; (2) unlike some of the exotic species imported by birds (Mukherjee et al. 2014), *H. juxtakochi* could easily find suitable host species in the US; and (3) as it continues its invasion of North America, *H. longicornis* may eventually be collected farther south, coinciding with *H. juxtakochi*'s existing range.

Materials and methods

Scanning electron microscopy (SEM)

Specimens of *H. longicornis* were obtained from US National Tick Collection archives for imaging. Males, females, and nymphs were sent from a laboratory colony started with specimens collected in Jeju-teukbyeoljachido, Republic of Korea (Accession # RML48803). Larvae originated from a colony started with specimens from Queensland, Australia (Accession # RML58949). Specimens were coated with gold and imaged with a JEOL JSM-6610LV scanning electron microscope (JEOL USA, Inc., Peabody, MA) (Figs 1–4). Larval and nymphal *H. juxtakochi* were collected by flagging in Guanacaste National Park, Costa Rica, and imaged in the same manner (Accession # USNMENT 986092).

Additional photomicrographs of *H. juxtakochi* (adult), *H. leporispalustris* (all stages) and *H. chordeilis* (all stages) were obtained from the US National Tick Collection archives (http://www.discoverlife.org).

Pictorial dichotomous key

A literature search was conducted and key characters useful for distinguishing the four species were gleaned from the following: Cooley (1946), Kohls (1960), Clifford et al. (1961), Fairchild et el. (1966), Hoogstraal et al. (1968), and Hoogstraal and Kim (1985). Of note, characters chosen to distinguish adult stages are present in both males and females of their respective species.

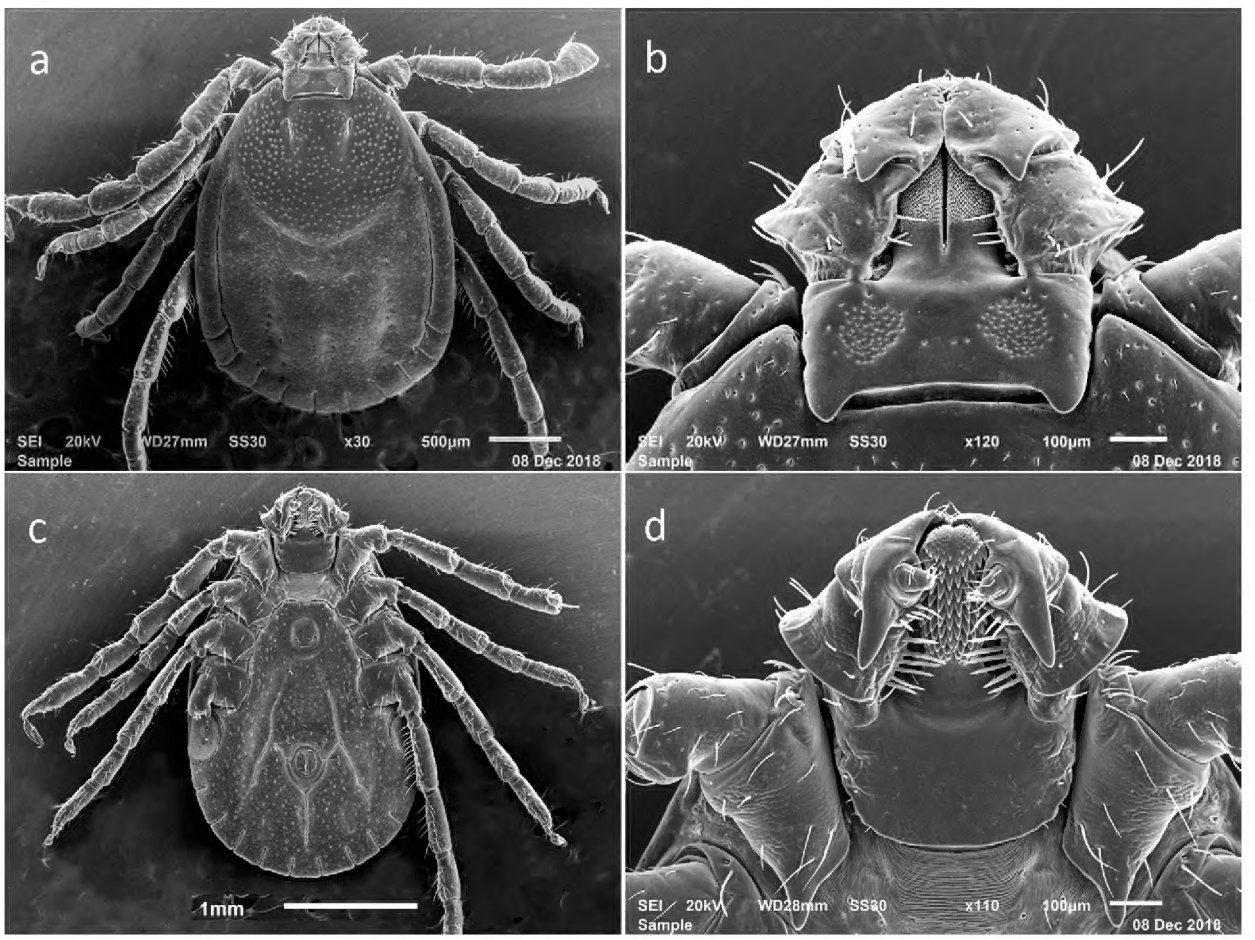


Figure 1. SEM photos of female *H. longicornis* from a colony started with specimens from Jeju-teukbyeoljachido, Republic of Korea (Accession # RML48803) **a** dorsal full body **b** dorsal capitulum **c** ventral full body **d** ventral capitulum.

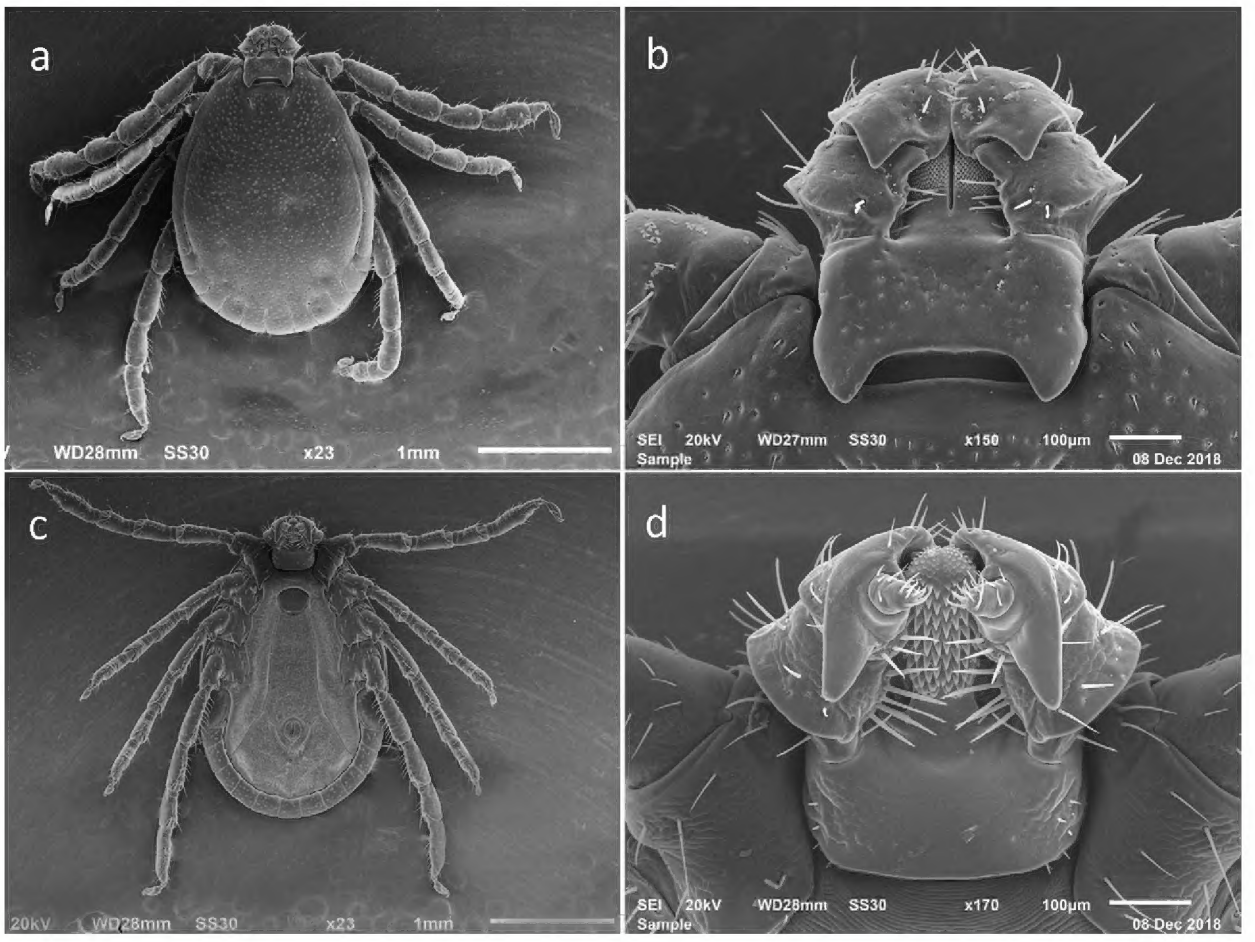


Figure 2. SEM photos of male *H. longicornis* from a colony started with specimens from Jeju-teukbyeoljachido, Republic of Korea. (Accession # RML48803) **a** dorsal full body **b** dorsal capitulum **c** ventral full body **d** ventral capitulum.

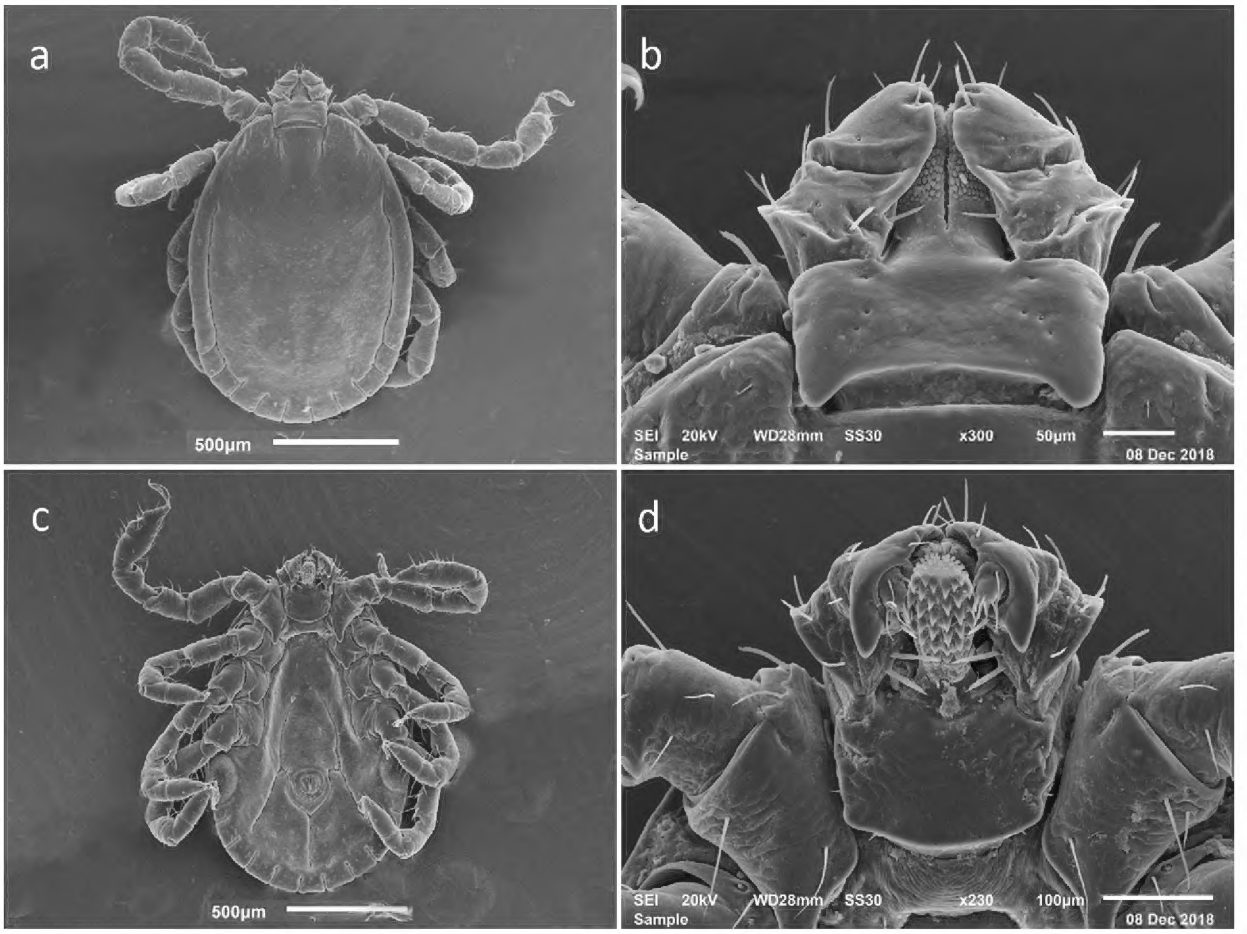


Figure 3. SEM photos of nymphal *H. longicornis* from a colony started with specimens from Jeju-teukbyeoljachido, Republic of Korea (Accession # RML48803). **a** dorsal full body **b** dorsal capitulum **c** ventral full body **d** ventral capitulum.

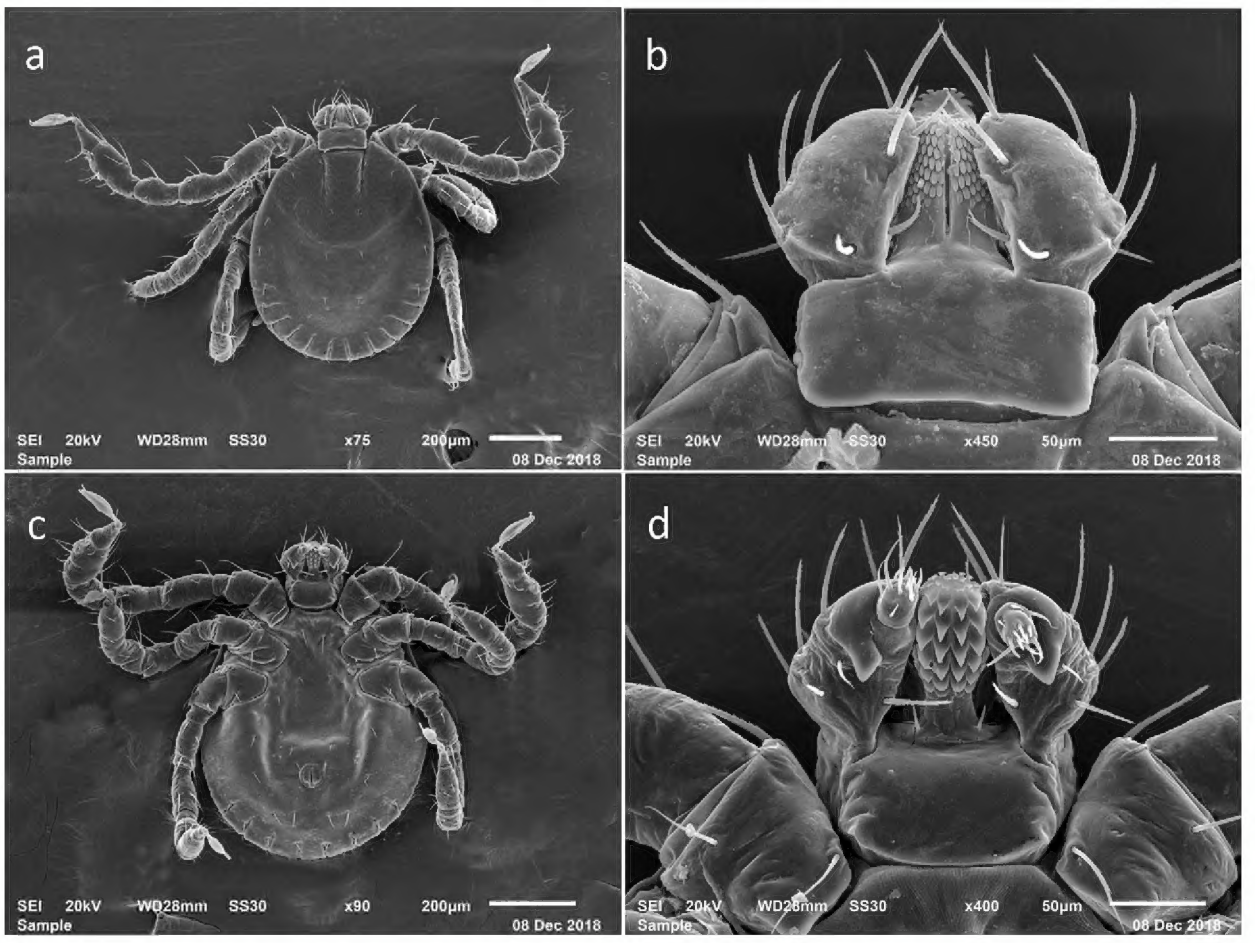


Figure 4. SEM photos of larval *H. longicornis* from a colony started with specimens from Queensland, Australia (Accession # RML58949). **a** dorsal full body **b** dorsal capitulum **c** ventral full body **d** ventral capitulum.

Key to *Haemaphysalis* spp. of North America

Adults (Fig. 5)

1 Palpal segment 3 dorsally with prominent retrograde spur (Fig. 5a) ..... ***Haemaphysalis* (*Kaiseriana*) *longicornis* Neumann, 1901**
– Palpal segment 3 without dorsal spur (Fig. 5b) **2**
2 Palpal segment 3 ventrally with long, slender, retrograde spur extending at least to middle of segment 2 (Fig. 5d) ..... ***Haemaphysalis* (*Gonixodes*) *juxtakochi* Cooley, 1946**
– Palpal segment 3 ventrally with short spur, not reaching segment 2 (Fig. 5c) **3**
3 Basis capituli ventrally with cornua at postero-lateral margins; dental formula 3/3 (Fig. 5e) ..... ***Haemaphysalis* (*Gonixodes*) *leporispalustris* (Packard, 1869)**
– Basis capituli ventrally without cornua; dental formula 5/5 (Fig. 5f) ..... ***Haemaphysalis* (*Aboimisalis*) *chordeilis* (Packard, 1869)**

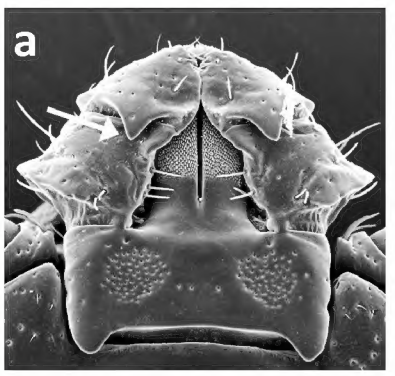
Nymphs (Fig. 6)

1 Basis capituli ventrally with cornua at postero-lateral margins (Fig. 6a) **2**
– Basis capituli ventrally without cornua at postero-lateral margins (Fig. 6b)..... **3**
2 Palpal segment 2 ventrally with 4–8 stout hairs along internal margin; palpal segment 3 ventrally with a short, blunt spur, not reaching anterior margin of segment 2 (Fig. 6c) ..... ***Haemaphysalis* (*Gonixodes*) *leporispalustris* (Packard, 1869)**
– Palpal segment 2 ventrally with 2 fine hairs along internal margin; palpal segment 3 ventrally with a longer, sharp, retrograde spur, extending to or beyond anterior margin of segment 2 (Fig. 6d) ..... ***Haemaphysalis* (*Gonixodes*) *juxtakochi* Cooley, 1946**
3 Dorsally, lateral margins of basis capituli straight (Fig. 6e); hypostomal dental formula 3/3..... ***Haemaphysalis* (*Kaiseriana*) *longicornis* Neumann, 1901**
– Dorsally, lateral margins of basis capituli pointed (Fig. 6f); hypostomal dental formula 2/2..... ***Haemaphysalis* (*Aboimisalis*) *chordeilis* (Packard, 1869)**

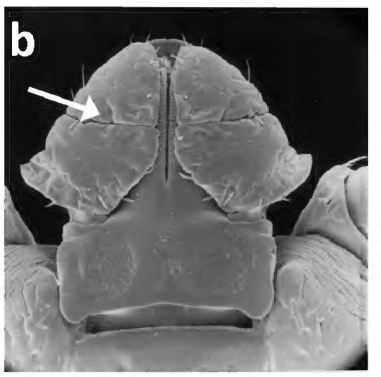
Larvae (Fig. 7)

1 Basis capituli ventrally with cornua at postero-lateral margins (Fig. 7a) **2**
– Basis capituli ventrally without cornua at postero-lateral margins (Fig. 7b) **3**
2 Basis capituli dorsally with prominent posteriorly directed cornua (Fig. 7c) ..... ***Haemaphysalis* (*Gonixodes*) *leporispalustris* (Packard, 1869)**
– Basis capituli dorsally with cornua faint or absent (Fig. 7d) ..... ***Haemaphysalis* (*Gonixodes*) *juxtakochi* Cooley, 1946**
3 Dorsally, lateral margins of basis capituli straight (Fig. 7e) ..... ***Haemaphysalis* (*Kaiseriana*) *longicornis* Neumann, 1901**
– Dorsally, lateral margins of basis capituli pointed (Fig. 7f) ..... ***Haemaphysalis* (*Aboimisalis*) *chordeilis* (Packard, 1869)**

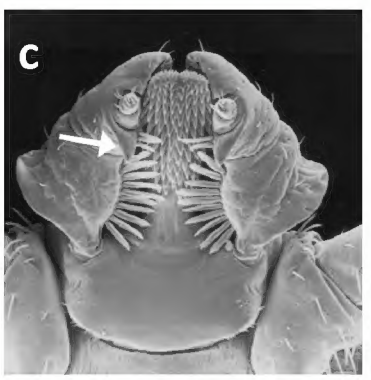
Dorsal spur on palpal segment 3, present or absent?



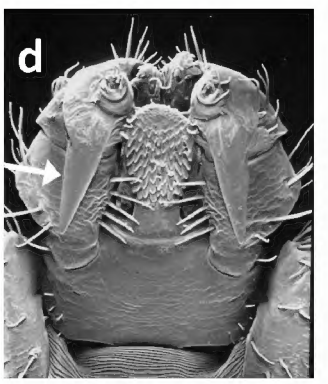
Present.....
H. longicornis



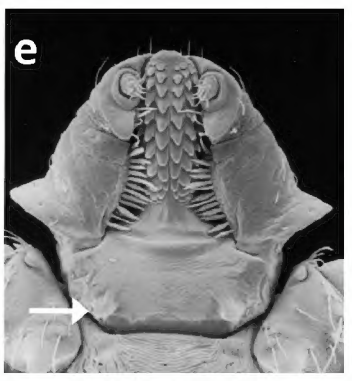
Absent.....
Ventral spur on palpal segment 3, long and pointed or short and blunt?



Short and blunt.....
Ventral cornua present or absent?



Long and pointed...
H. juxtakochi

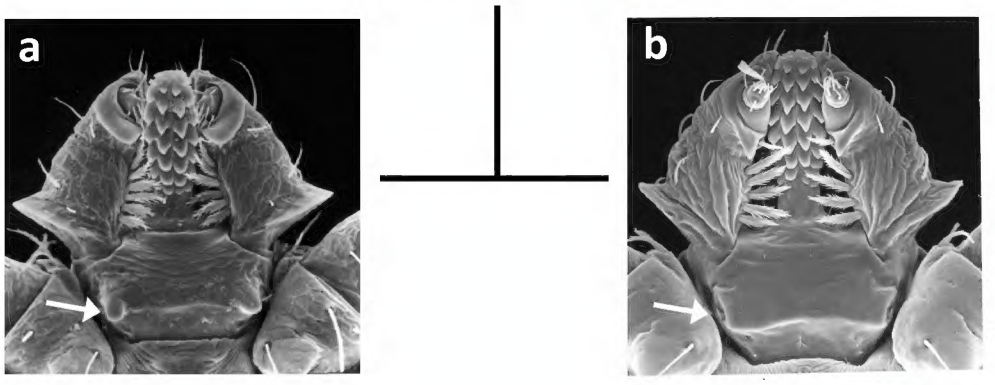


Present.....
H. leporispalustris

Absent.....
H. chordeilis

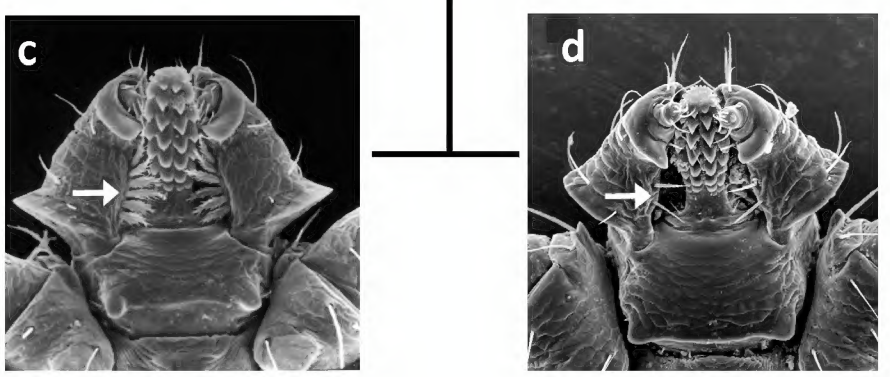
Figure 5. Pictorial key to adults of *Haemaphysalis* spp. occurring in North America.

Ventral cornua, present or absent?



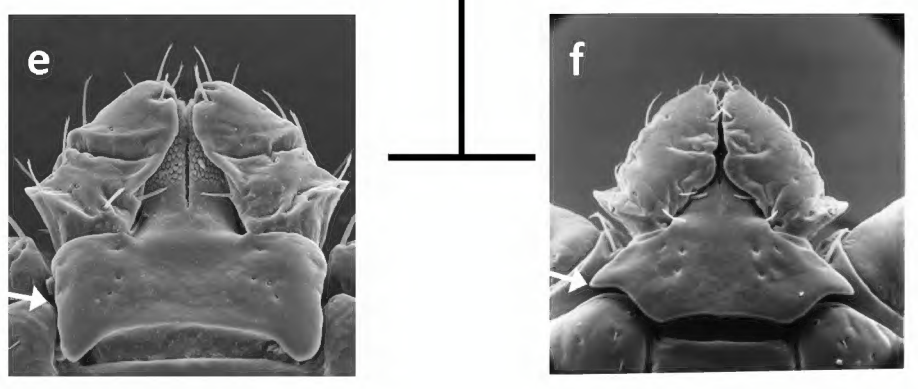
Present.....
Internal margin of palpal segment 2, 4-8 stout hairs or 2 fine hairs?

Absent.....
Lateral margins of dorsal basis capituli, straight or pointed?



4-8 stout hairs.....
H. leporispalustris

2 fine hairs.....
H. juxtakochi

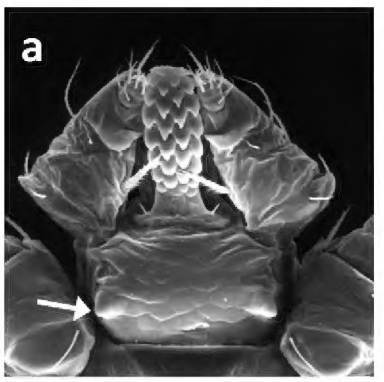


Straight.....
H. longicornis

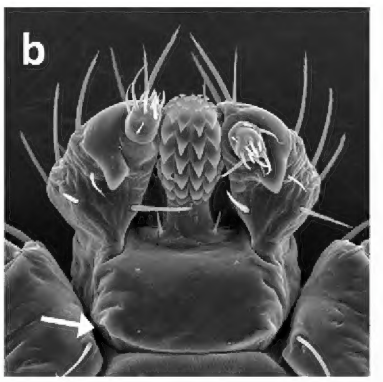
Pointed.....
H. chordeilis

Figure 6. Pictorial key to nymphs of *Haemaphysalis* spp. occurring in North America.

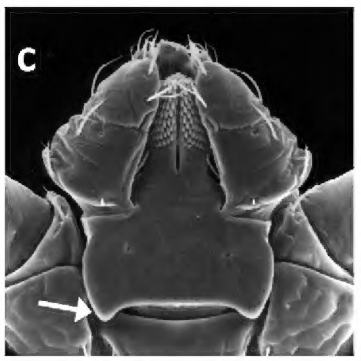
Ventral cornua, present or absent?



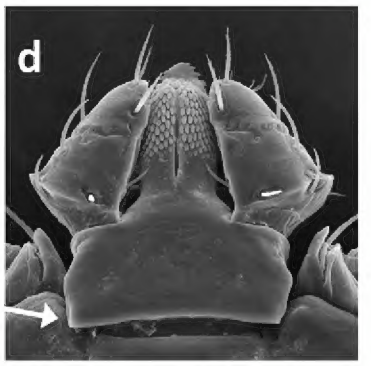
Present.....
Dorsal cornua prominent or absent?



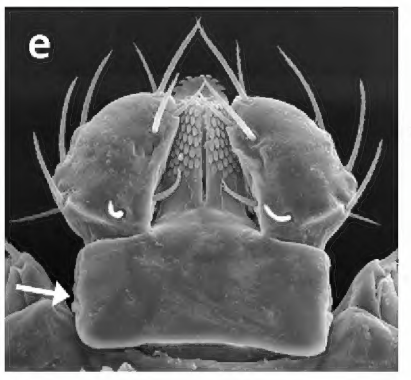
Absent.....
Lateral margins of dorsal basis capituli, straight or pointed?



Prominent dorsal cornua.....
H. leporispalustris



Dorsal cornua faint or absent.....
H. juxtakochi



Straight.....
H. longicornis

Pointed.....
H. chordeilis

Figure 7. Pictorial key to larvae of *Haemaphysalis* spp. occurring in North America.

Conclusions

This key enables researchers to distinguish the four species of *Haemaphysalis* that may be encountered in North America in all life stages. Previously, readers would have had to peruse keys from several distinct parts of the world in order to compare the morphology of these four species, e.g. US keys containing *H. chordeilis* (Furman and Loomis 1984, Keirans and Litwak 1989); Old World keys with *H. longicornis*, including Japan (Yamaguti et al. 1971) and Australia (Roberts 1970, Barker and Walker 2014); and Central and South American keys for *H. juxtakochi* (Fairchild et al. 1966, Nava et al. 2017).

The ability to easily distinguish these four species will contribute to ongoing efforts to map the distribution of *Haemaphysalis longicornis* in North America and understand the potential risks posed by this recently discovered exotic tick species (Rainey et al. 2018). This tool will also help to improve our understanding of the biology and ecology of native *Haemaphysalis* spp., which have been relatively poorly studied compared to other native ixodids, and will promote the early detection of any northward expansions of *H. juxtakochi*. In this manner we can capitalize on the interest generated by the arrival of *H. longicornis* to augment our understanding of the existing New World haemaphysaline fauna.

However, as *Haemaphysalis* is the second largest genus in the tick family Ixodidae (so-called hard ticks), with over 160 additional species in the Old World (Petney et al. 2007, Guglielmone et al. 2014), including important disease vectors (de la Fuente et al. 2008), careful monitoring to detect the potential arrival of other members of this genus is encouraged. Should additional *Haemaphysalis* species establish themselves in North America, this key will require revision.

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